

We Claim:

1. Novel temperature regulated promoters having SEQ ID No.1, designated as *nmt-185* and SEQ ID No.2, designated as *nmt-146*.
- 5 2. Promoters as claimed in claim 1, wherein the promoters have been isolated from *Schizosaccharomyces pombe*.
3. Promoters as claimed in claim 1, wherein GFP expression of said promoters is about 95 % within 3 hrs.
4. Promoters as claimed in claim 3, wherein GFP expression of said promoters
10 is about 91.4 % within 3 hrs.
5. Promoters as claimed in claim 1, wherein said promoters have β -galactosidase activity of about 150 ± 20 units within 3 hrs of induction.
6. Promoters as claimed in claim 1, wherein said promoters have β -galactosidase activity of about 124.3 ± 20 units within 3 hrs of induction.
- 15 7. Promoters as claimed in claim 1, wherein said promoters have maximum specific activity of about 900 I.U/mg in 3 hrs.
8. Promoters as claimed in claim 7, wherein said promoters have maximum specific activity of about 870 ± 16 I.U/mg in 3 hrs.
9. Promoters as claimed in claim 1, wherein said promoters enhance expression
20 of *cdc-18* gene within 3 hrs of induction.
10. Promoters as claimed in claim 1, wherein said promoters give lower leaky expression of proteins.
11. Promoters as claimed in claim 1, wherein said promoters are not deleterious to the cell viability.
- 25 12. Promoters as claimed in claim 1, wherein said promoters reduce the level of proteolytic degradation.
13. Novel temperature regulated expression vectors having Accession No. MTCC 5106 and MTCC 5107 deposited at International depository of Institute of Microbial Technology (IMTECH), Chandigarh, India, wherein,
30 (a) expression vector having Accession MTCC 5106 is harbouring temperature regulated promoter having SEQ ID No.1, designated as *nmt-185* and
(b) expression vector having Accession No. MTCC 5107 is harbouring

temperature regulated promoter having SEQ ID No. 2, designated as
nmt-146

14. Vectors as claimed in claim 13, wherein the promoters of the said vectors have been isolated from *Schizosaccharomyces pombe*.
- 5 15. Vectors as claimed in claim 13, wherein said vectors have GFP activity of about 95 % within 3 hrs.
16. Vectors as claimed in claim 15, wherein said vectors have GFP activity of about 91.4 % within 3 hrs.
- 10 17. Vectors as claimed in claim 13, wherein said vectors have β -galactosidase activity of about 150 ± 20 units within 3 hrs of induction.
18. Vectors as claimed in claim 17, wherein said vectors have β -galactosidase activity of about 124.3 ± 20 units within 3 hrs of induction.
19. Vectors as claimed in claim 13, wherein said vectors have maximum specific activity of about 900 I.U/mg in 3 hrs.
- 15 20. Vectors as claimed in claim 13, wherein said vectors have maximum specific activity of about 870 ± 16 I.U/mg in 3 hrs.
21. Vectors as claimed in claim 13, wherein said vectors enhance expression of *cdc-18* gene within 3 hrs of induction.
22. Vectors as claimed in claim 13, wherein said vectors give lower leaky expression of proteins.
- 20 23. Vectors as claimed in claim 13, wherein said vectors are not deleterious to the cell viability.
24. Vectors as claimed in claim 13, wherein said vectors reduce the level of proteolytic degradation.
- 25 25. A process of isolating novel temperature regulated promoters from *Scizosaccharomyces pombe* said process comprising the steps of:
 - (a) constructing a partial genomic DNA library with restriction enzyme *Sau3AI*, to obtain partial genomic DNA sequences in the range of about 100 bp to 2000bp,
 - 30 (b) ligating the genomic DNA library sequences of step (a) with vector pGFP without a promoter
 - (c) transforming the vector of step (b) to *S. pombe* strain,

- (d) screening of *S. pombe* strain containing the promoter library,
- (e) isolating and identifying two clones of (step d) by stimulating GFP expression,
- 5 (f) using the clones obtained in step (e) to check repress or express of GFP expression by temperature shift,
- (g) sequencing the genomic DNA fragments of (f) as new promoter elements having SEQ ID No. 1 and SEQ ID No.2, designating the promoters as *nmt-185* and *nmt-146*, useful as promoters, and
- 10 (h) cloning the said promoter elements into the novel vectors having Accession nos. MTCC 5106 and 5107 respectively.
26. A process as claimed in claim 25, wherein the step (f) the temperature shifts are 25°C and 37°C.
27. A process as claimed in claim 25, wherein the promoters have been isolated
- 15 from *Schizosacchromyces pombe*.
28. A process as claimed in claim 25, wherein the sequence of the said promoter element *nmt-185* and *nmt-146* is identical or more than 80% homologous to the sequence of *nmt1*.
29. A process as claimed in claim 25, wherein the promoter element *nmt-185* and
- 20 *nmt-146* are repressed in the temperature range of about 33° to 37°C.
30. A process as claimed in claim 25, wherein the promoter element *nmt-185* and *nmt-147* are expressed in the temperature range of about 22° to 28°C.
31. A process as claimed in claim 25, wherein the promoter element *nmt- 185* is about 185 bases long.
- 25 32. A process as claimed in claim 25, wherein the promoter element *nmt- 146* is only 146 bases long.
33. A process as claimed in claim 25, wherein the promoter elements *nmt-186* and *nmt-145* can express or repress the genes GFP, Streptokinase, β -galactosidase and *cdc18 gene*.
- 30 34. A process as claimed in claim 25, wherein GFP expression of said promoters is about 95 % within 3 hrs.

35. A process as claimed in claim 34, wherein GFP expression of said promoters is about 91.4 % within 3 hrs.
36. A process as claimed in claim 25, wherein said promoters have β -galactosidase activity of about 150 ± 20 units within 3 hrs of induction.
- 5 37. A process as claimed in claim 36, wherein said promoters have β -galactosidase activity of about 124.3 ± 20 units within 3 hrs of induction.
38. A process as claimed in claim 25, wherein said promoters have maximum specific activity of about 900 I.U/mg in 3 hrs.
39. A process as claimed in claim 38, wherein said promoters have maximum
10 specific activity of about 870 ± 16 I.U/mg in 3 hrs.
40. A process as claimed in claim 25, wherein said promoters enhance expression of *cdc-18* gene within 3 hrs of induction.
41. A process as claimed in claim 25, wherein said promoters give lower leaky expression of proteins.
- 15 42. A process as claimed in claim 25, wherein said promoters are not deleterious to the cell viability.
43. A process as claimed in claim 25, wherein said promoters reduce the level of proteolytic degradation.
44. A process of preparing novel expression vectors based temperature regulated
20 novel promoter elements isolated from *Scizosaccharomyces pombe* said process comprising steps of:
- (a) constructing a partial genomic DNA library with restriction enzyme *Sau3AI*, to obtain partial genomic DNA sequences in the range of about 100 bp to 2000bp,
 - 25 (b) ligating the genomic DNA library sequences of step (a) with vector pGFP without a promoter
 - (c) transforming the vector of step (b) to *S.pombe* strain,
 - (d) screening of *S. pombe* strain containing the promoter library,
 - (e) isolating and identifying two clones of (step d) by stimulating GFP
30 expression,
 - (f) using the clones obtained in step (e) to check repress or express of GFP expression by temperature shift,

- (g) sequencing the genomic DNA fragments of (f) as new promoter elements of 185 bases having SEQ ID No.1 and 146 bases having SEQ ID No.2, designated as *nmt-185* and *nmt-146* respectively, and
- (h) cloning the said promoter elements into the novel vectors having
5 Accession vector nos. MTCC 5106 and 5107 respectively.
45. A process as claimed in claim 44, wherein the step (f) the temperature shifts are 25°C and 37°C.
46. A process as claimed in claim 44, wherein the promoters have been isolated from *Schizosacchomyces pombe*.
- 10 47. A process as claimed in claim 44, wherein the sequence of the said promoter element *nmt-185* and *nmt-146* is identical or more than 80% homologous to the sequence of *nmt1*.
48. A process as claimed in claim 44, wherein the promoter element *nmt-185* and *nmt-146* are repressed in the temperature range of about 33° to 37°C.
- 15 49. A process as claimed in claim 44, wherein the promoter element *nmt-185* and *nmt-147* are expressed in the temperature range of about 22° to 28°C.
50. A process as claimed in claim 44, wherein the promoter element *nmt- 185* is about 185 bases long.
51. A process as claimed in claim 44, wherein the promoter element *nmt- 146* is
20 only 146 bases long.
52. A process as claimed in claim 44, wherein the promoter elements *nmt-186* and *nmt-145* can express or repress the genes GFP, Streptokinase, β -galactosidase and *cdc18 gene*.
53. A process as claimed in claim 44, wherein said vectors have GFP activity of
25 about 95 % within 3 hrs.
54. A process as claimed in claim 53, wherein said vectors have GFP activity of about 91.4 % within 3 hrs.
55. A process as claimed in claim 44, wherein said vectors have β -galactosidase activity of about 150 ± 20 units within 3 hrs of induction.
- 30 56. A process as claimed in claim 55, wherein said vectors have β -galactosidase activity of about 124.3 ± 20 units within 3 hrs of induction.

57. A process as claimed in claim 44, wherein said vectors have maximum specific activity of about 900 I.U/mg in 3 hrs.
58. A process as claimed in claim 57, wherein said vectors have maximum specific activity of about 870 ± 16 I.U/mg in 3 hrs.
- 5 59. A process as claimed in claim 44, process as claimed in claim 24, wherein said vectors enhance expression of *cdc-18* gene within 3 hrs of induction.
60. A process as claimed in claim 59, wherein said vectors give lower leaky expression of proteins.
61. A process as claimed in claim 44, wherein said vectors are not deleterious to
10 the cell viability.
62. A process as claimed in claim 44, wherein said vectors reduce the level of proteolytic degradation.